This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

THIS PAGE BLANK (USPTO)

As discussed in the description

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
6 December 2001 (06.12.2001)

PCT

(10) International Publication Number
WO 01/92908 A1

(51) International Patent Classification[7]: G01R 33/483

(21) International Application Number: PCT/NO01/00220

(22) International Filing Date: 28 May 2001 (28.05.2001)

(25) Filing Language: Norwegian

(26) Publication Language: English

(30) Priority Data:
20002853 2 June 2000 (02.06.2000) NO

(71) Applicant and
(72) Inventor: SØRLAND, Geir, H. [NO/NO]; Hagebyveien 32, N-9404 Harstad (NO).

(74) Agent: RUDI, Alf-Petter; P.O. Box 2459, N-9272 Tromsø (NO).

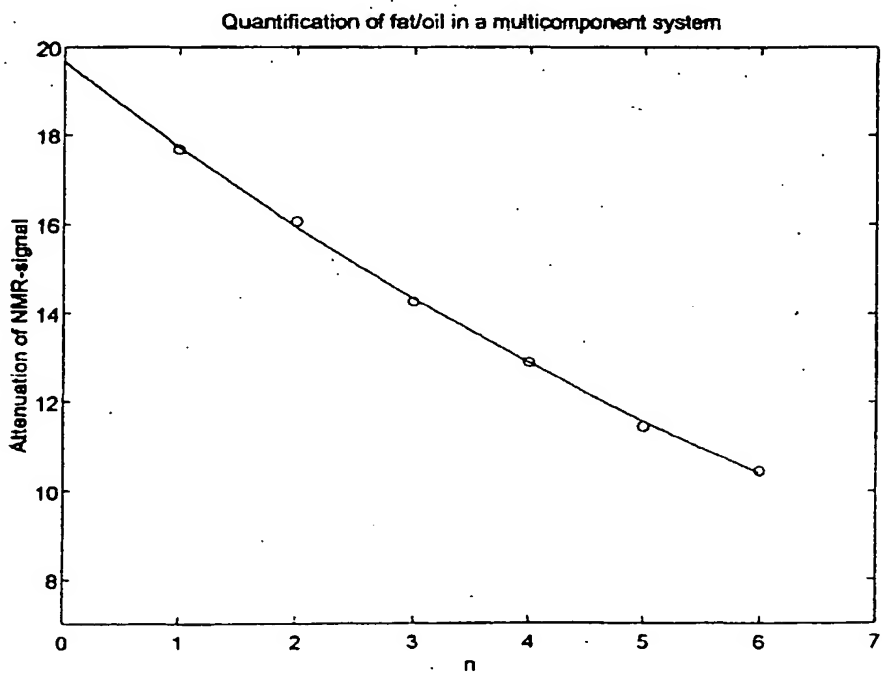
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: A METHOD FOR MEASURING THE CONTENT OF FAT/OIL IN A MULTI COMPONENT SYSTEM



(57) Abstract: The invention is a method for determination of the content of fat in a multicomponent system. The method applies nuclear magnetic resonance (NMR) for the determination of fat/oil in for example fillets of fish, olives, paint or ice cream.

WO 01/92908 A1

A Method for measuring the content of fat/oil in a multi component system

The invention is a method for determining the amount of fat in a multicomponent system where water, sugar, protein or other components containing hydrogen maybe present. The method applies nuclear magnetic resonance (NMR) for determining the amount of fat/oil in for example fish, olives, paints, or dairy products.

There are several methods used for determining the content of fat/oil; In the Fosslet-, Soxhlet-, and Ethylacetate methods one adds solvents which extracts the fat/oil. By removing the solvent, one is left with the fat/oil which originally was found in the sample. These methods require well trained laboratory personnel and the use of toxic solvent which may damage the environment.

Another way to measure the fat/oil content is to use Near Infrared Reflectance (NIR) spectroscopy. This type of measurements require a lot of calibration work for a given system as the method is very sensitive to changes in the texture of the sample.

The purpose of the proposed method is to supply a robust method for determination of fat/oil in a generally multicomponent system. The method is fats, accurate, and it does not demand laboratory personnel who has specific knowledge with respect to the method.

This is achieved by placing the sample to be investigated in a homogeneous/static magnetic field, and being exposed to an oscillating magnetic field which in combination with a magnetic field gradient over the sample measures the nuclear magnetic moment of the protons, in a multipulsed field gradient spin-echo experiment (m-PFGSE), as one resolves the fat/oil-signal from the other components by their significant difference in mobility and/or transverse relaxation times.

Further details on the invention will be apparent in the following description of the method with reference to the figures.

Figure 1. A multicomponent sample placed in a NMR-based spectrometer.

Figure 2. Signal arising from the use of a multi-Pulsed magnetic Field Gradient Spin-Echo method (m-PFGSE).

Figure 3. Result from a m-PFGSE on homogenised filet of herring, which shows the attenuation of fat/oil from a m-PFGSE due to mobility and T2*.

When placing hydrogen in an external magnetic field, the nuclear magnetic moment will align towards the direction of this field. The Hamiltonian for noninteracting nuclear magnetic spins in an external magnetic field can be written

$$\mathrm{H} = -\gamma\, \hbar I H_{(t)} \qquad \text{(L1)}$$

where γ=gyromagnetic ratio , $\hbar$=Planck's constant. I=spin operator and H(t) = external magnetic field. The time dependency of $H_{(t)}$ is included in order to make (L1) valid when the system is influenced by an oscillating magnetic field (RF-field) and magnetic field gradients (g). When the Hamiltonian, $H_{(t)}$, is constant and homogeneous (=H_0), the eigenvalues, the energy levels, of the hydrogen's nuclear spin may be written

$$E = \pm\frac{1}{2}\gamma\, \hbar H_0 = \pm\frac{1}{2}\hbar\omega_0 \qquad \text{(L2)}$$

The difference between the to energy levels is thus written

$$\Delta E = \hbar\omega_0 \qquad \text{(L3)}$$

In thermal equilibrium a difference population between upper and lower level is given by the Boltzmann factor

$$\frac{n_{upper}}{n_{lower}} = e^{-\frac{\hbar\omega}{kT}} \qquad \text{(L4)}$$

where T is absolute temperature and k= Boltzmann's constant. The difference in population will generate a net nuclear magnetic moment which will depend on the content of hydrogen/proton. In thermal equilibrium the moment will be aligned with the external magnetic field. By imposing an oscillating magnetic field, RF-field, transverse to the external magnetic field Ho, transitions between the energy levels will occur (ref.1). The direction of the net nuclear magnetic moment will then move away from thermal equilibrium with the external field. When the RF-field is switched off, the system will

characteristic relaxation times T1 (longitudinal relaxation) and T2 (transverse relaxation).

The path back to thermal equilibrium in combination with an oscillating net nuclear magnetic moment transverse to Ho, will cause changes in the magnetic flux which can be recorded with the same RF-coil which was used to excite the system. The current induced in the coil will then be proportional to the number of hydrogen in the system, and from the intensity of the signal one may quantify the content of hydrogen in the system (figure 1).

One may record the mobility of the hydrogen by making use of a magnetic field gradient. This magnetic field gradient, g, imposes a position dependent frequency an the system, and with which the nuclear magnetic moment of the proton is oscillating in a plane transverse to H_o

$$\omega = \gamma H_0 + \gamma g z \qquad \text{(L5)}$$

By using RF-pulses and magnetic field gradients in a NMR-diffusion experiment (ref.2), there is a dephasing of the net magnetic moment given by

$$\varphi = \gamma g (z_2 - z_1) \qquad \text{(L6)}$$

(Z_2-Z_1) is the distance the protons has moved during the NMR-diffusion experiment. For larger values on the mobility (Z_2-Z_1), the induced current in the RF-coil, the NMR-signal, will decrease because of the dephasing.
When assuming a Gaussian distribution of diffusivities and monoexponential attenuation of the NMR-signal due to relaxation processes, the attenuation of the NMR-signal is written

$$I = I_0 e^{-\frac{t_1}{T_2}} e^{-\frac{t_2}{T_1}} e^{-\gamma^2 g^2 D \int_0^t (\int_0^{t'} g(t'')dt'')^2 dt'} \qquad \text{(L7)}$$

t_1 = duration the NMR-signal is influenced by transverse relaxation processes
t_2 = duration the NMR-signal is influenced by longitudinal relaxation processes
g(t'') = total magnetic field gradient, external and internal.

D = diffusion coefficient
T_1 = Characteristic longitudinal relaxation time
T_2 = Characteristic transverse relaxation time
Io = Initial intensity of the NMR-signal

There are several ways to perform a diffusion experiment by NMR. Here a so-called multi-pulsed magnetic field gradient spin echo experiment is applied (m-PFGSE) (see figure 2). Figure 2 displays the monopolar version. With this sequence it becomes unnecessary to perform extra correction for longitudinal relaxation processes, transverse relaxation processes, and the NMR-signal will be refocused with respect to internal magnetic field gradients. In addition, the uncertainty due to eddy current field is minimised as one is using the same gradient strength throughout the experiment.

The echo-attenuation for the m-PFGSE-sequence in figure 2 is written

$$I = I_0 e^{-n\bullet[\frac{2\tau}{T_2} - \frac{2\tau^3}{3}\gamma^2 G_i^2 D]} e^{-n\bullet[\gamma^2 g^2 D\delta^2(\tau - \frac{\delta}{3})]} \qquad \text{(L8)}$$

Gi is the internal magnetic field gradient caused by changes in magnetic susceptibilities throughout the sample, g is the externally applied magnetic field gradient, δ is the gradient pulse length, and τ is the time interval between 90-degree RF-pulse and 180-degree RF-pulse.

By defining the unknown parameter

$$K = \frac{2\tau}{T_2} + \frac{2\tau^3}{3}\gamma^2 G_i^2 D + \gamma^2 g^2 D\delta^2(\tau - \frac{\delta}{3}) \qquad \text{(L9)}$$

the attenuation may be written

$$I = I_0 e^{-n\bullet K} \qquad \text{(L10)}$$

Terms including relaxation, diffusion due to internal magnetic field gradients and diffusion terms due to applied magnetic field gradients, are thus collected as one unknown, K.

To separate between NMR-signal from fat/oil and the other components, one makes use of the difference in mobility and transverse relaxation time. Fat/oil has significant different mobility from water and sugar dissolved in water. By fitting the applied field gradient pulse such that water signal and possible signal from sugar dissolved in water is suppressed at the first echo, then the m-PFGSE-experiment can be used to quantify the fat(oil) directly. Due to the very short transverse relaxation times (< 1ms) of protein and solid sugar, their NMR-signal will not contribute when the first measuring point (n=0) in the m-PFGSE experiment is at 5 ms or more. The attenuation can then be written

$$I = I_{fat} e^{-n \bullet K_{fat}} \qquad \text{(L11)}$$

A weighted linear fit of the logarithm of L11 to the function

$$y = -ax + c \qquad \text{(L12)}$$

yields a value for c where

$$I_{fat} = e^{c} \qquad \text{(L13)}$$

By weighting the fit one takes into consideration that the model in (L11) is not valid at all times. When the observation time approaches 0 (n ->0), the validity will increase. The first measuring points are therefore given more weight than the last ones.

Diffusion and relaxation effects in the NMR-signal is now corrected for, and the signal is meant to a measure for the content of fat(oil) on the sample.

The method is tested on homogenised salmon, herring and mackerel. Typical experimental results for homogenised herring is shown in figure 3. Control measurements have been performed using ethylacetate as solvent in an extraction method of fat/oil. The results from the two different methods are found in table 1.

	Fat content by the NMR-method / %	Fat content by extraction / %
Wild salmon	5.5 +/- 0.1	5.3
Bred salmon	11.1 +/- 0.2	10.9
Herring	17.2 +/- 0.2	16.9
Mackerel	30.0 +/- 0.4	29.8

Table 1: NMR-results for fat content in different types of fish compared with an extraction method.

References

Ref.1: *NMR-Signal Reception: Virtual Photons and Coherent Spontaneous Emission*, Concepts Magnetic Resonance 9: 277-297 (1997).

Ref.2: *Pulsed-Field Gradient Nuclear Magnetic Resonance as a Tool for Studying Translational Diffusion: Part 1. Basic Theory*, Concepts Magnetic Resonance 9: 299-336 (1997).

Ref. 3: *A review of H nuclear magnetic resonance relaxation in pathology: Are T1 and T2 diagnostics?*, Medical Physics 14 (1), Jan/Feb 1987.

Claims

1. A way to measure the content of fat or oil in a multicomponent system, **characterised by** a sample placed in a homogenous/static magnetic field and affected by an oscillating magnetic field (figure 1), which together with a magnetic field gradient measures the nuclear magnetic moment of the protons, in a multipulsed diffusion/relaxation experiment (figure 2), as one directly resolves the fat signal from the other components due to their differences in mobility and characteristic relaxivity.

2. A way to measure the fat or oil content in a multicomponent system according to **1, characterised by** a simultaneous correction for diffusion- and relaxation effects in the fat signal using a multipulsed diffusion/relaxation experiment (figure 2), by fitting the experimental result as a function of number of echoes, as the fitted signal at number of echo=0 will express the fat content.

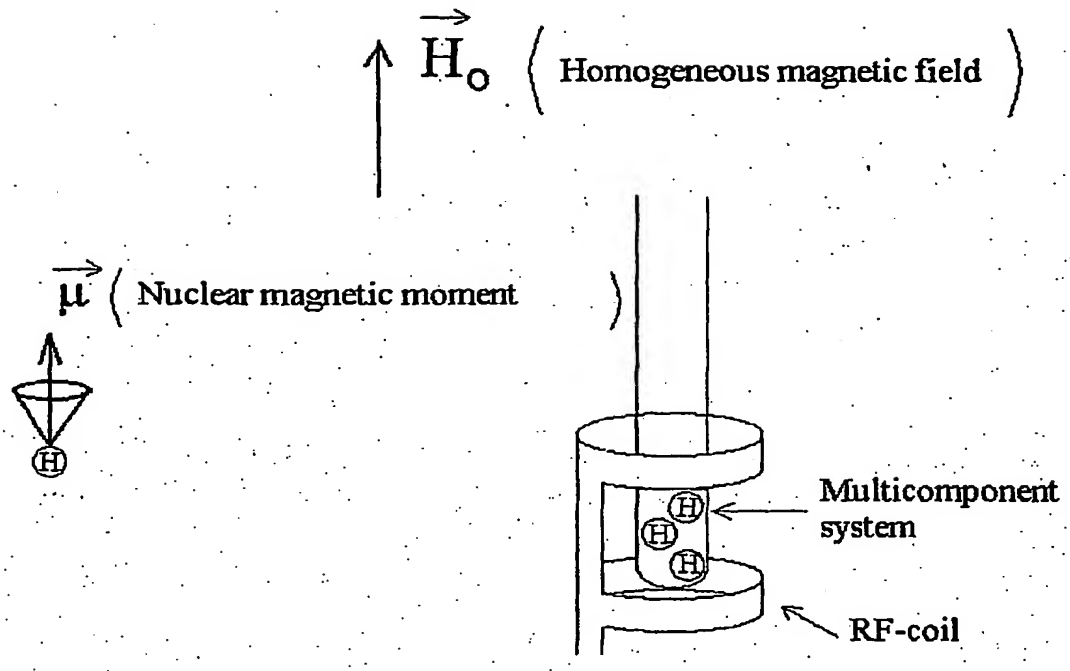

$\vec{H}_o$
Homogeneous magnetic field
$\vec{\mu}$
Nuclear magnetic moment
H
Multicomponent system
RF-coil

Figure 1

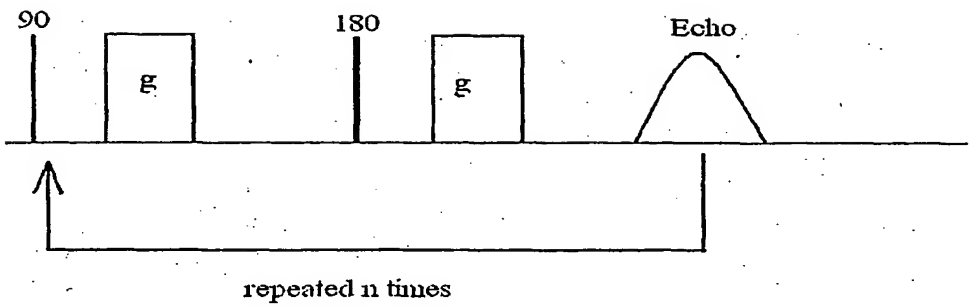


Figure 2

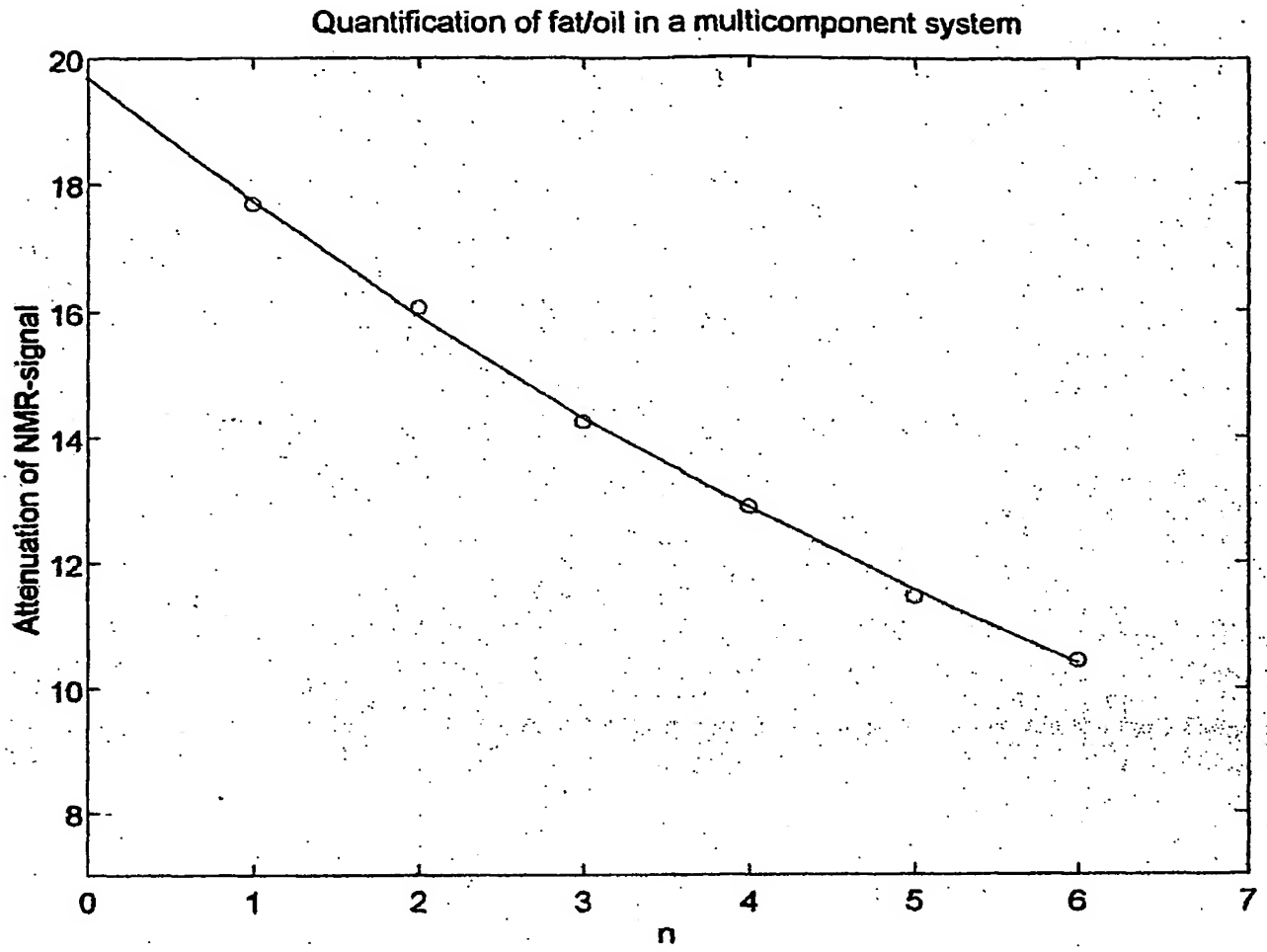

Quantification of fat/oil in a multicomponent system
Attenuation of NMR-signal
n
20
18
16
14
12
10
8
0
1
2
3
4
5
6
7

Figure 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NO 01/00220

A. CLASSIFICATION OF SUBJECT MATTER

IPC7: G01N 33/483
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7: G01N, G01R

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4785245 A (H.S.LEW ET AL), 15 November 1988 (15.11.88), abstract ---	1-2
Y	WO 9954751 A1 (SØRLAND,GEIR,H.), 28 October 1999 (28.10.99), see the whole document --- --------	1-2

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
16 October 2001	17 -10- 2001
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Mats Raidla /itw Telephone No. +46 8 782 25 00

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Information on patent family members

01/10/01

International application No.
PCT/NO 01/00220

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4785245 A	15/11/88	NONE	
WO 9954751 A1	28/10/99	AU 3175799 A	08/11/99
		NO* 981517 A	04/10/99

THIS PAGE BLANK (USPTO)